

Using plant traits to understand the contribution of biodiversity effects to annual crop community productivity

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Citation: Engbersen, N., L. Stefan, R. W. Brooker, and C. Schöb. 2022. Using plant traits to understand the contribution of biodiversity effects to annual crop community productivity. *Ecological Applications* 32(1):e02479. 10.1002/eap.2479

Abstract. Increasing biodiversity generally enhances productivity through selection and complementarity effects not only in natural, but also in agricultural, systems. However, the quest to explain why diverse cropping systems are more productive than monocultures remains a central goal in agricultural science. In a mesocosm experiment, we constructed monocultures, two- and four-species mixtures from eight crop species with or without fertilizer and both in temperate Switzerland and dry, Mediterranean Spain. We measured physical factors and plant traits and related these in structural equation models to selection and complementarity effects to explain seed yield differences between monocultures and mixtures. Increased crop diversity increased seed yield in Switzerland. This positive biodiversity effect was driven to almost the same extent by selection and complementarity effects, which increased with plant height and specific leaf area (SLA), respectively. Also, ecological processes driving seed yield increases from monocultures to mixtures differed from those responsible for seed yield increases through the diversification of mixtures from two to four species. Whereas selection effects were mainly driven by one species, complementarity effects were linked to larger leaf area per unit leaf weight. Seed yield increases due to mixture diversification were driven only by complementarity effects and were not mediated through the measured traits, suggesting that ecological processes beyond those measured in this study were responsible for positive diversity effects on yield beyond two-species mixtures. By understanding the drivers of positive biodiversity–productivity relationships, we can improve our ability to predict species combinations that enhance ecosystem functioning and can promote sustainable agricultural production.

Key words: agroecology; biodiversity effects; biodiversity–productivity; complementarity effects; crop mixtures; intercropping; plant traits; selection effects.

INTRODUCTION

Plant primary productivity increases with higher species diversity in seminatural grasslands (e.g., Tilman et al. 2001, Cardinale et al. 2006). While the majority of research in this area has been done in perennial systems (Tilman et al. 2001, Huang, 2018), recent studies have demonstrated similar effects in annual systems (Li et al. 2014, Brooker, 2015, Stomph, 2020). Intercropping of annual crops, in which at least two crop species are grown in close proximity at the same time, is therefore a promising application of agroecological concepts. By making use of beneficial aboveground and belowground species interactions, intercropping can lead to overyielding, which is the increased yield in a mixture compared with the average of the monocultures (Vandermeer 1989).

Two main mechanisms have been proposed to explain positive biodiversity–productivity relationships. First, sampling or selection effects (SE) encompass the greater probability that more diverse communities include highly productive species or functional groups, which then account for the majority of productivity (Tilman and Lehman 1997). Enhanced ecosystem functioning in diverse agroecosystems can be driven by SE (i.e., communities with more species are more likely to host a high-performing species). For instance, in China, a hot-spot of intercropping, a recent meta-analysis has shown that 10% of all yield gain of intercropping compared with sole cropping were due to SE (Li et al. 2020).

The second mechanism is the complementarity effect (CE), caused through resource partitioning or facilitation. Resource partitioning involves more diverse communities containing species with contrasting demands on resources, which leads to a more complete exploitation of available resources in diverse plant communities compared with monocultures and therefore increased productivity (Tilman et al. 1997, Loreau and Hector 2001). The partitioning of resources can occur across

Manuscript received 30 September 2020; revised 15 June 2021; accepted 8 July 2021. Corresponding Editor: Fabian D. Schneider.

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spatial, temporal or chemical gradients. Belowground, different rooting depths allow plants to take up water or nutrients from different soil layers, therefore limiting competition. While this has been observed for water uptake (Miyazawa et al. 2009), the evidence of resource partitioning for soil nutrients as a driver of biodiversity effects is less clear (Von Felten et al. 2012, Jesch, 2018). Aboveground, diverse communities can harbor more diverse canopy growth forms allowing for a more complete use of photosynthetically active radiation (PAR) (Spehn, 2002, Fridley 2003), leading in some cases to yield advantages in mixtures compared with monocultures (Bedoussac and Justes 2010).

Facilitation involves plants altering their environment in a way that is beneficial to at least one co-occurring species (Brooker, 2008). and can happen via either the enrichment of resource pools by one plant and also benefiting neighboring plants or the mediation of physical stress. The facilitative benefits of legumes increasing soil N for their neighbors by using atmospheric N₂ as their N source are well known, especially for cereals intercropped with legumes (Spehn et al. 2002, Temperton et al. 2007). Other belowground facilitative mechanisms shown to occur in intercrops include the enrichment of resource pools by hydraulic lift, which not only facilitates water uptake (Sekiya and Yano 2004), but can also enhance nutrient mobilization and lead to increased nutrient status of the intercrop (Sun et al. 2013). Other evidence of facilitation mediating physical stress in crop systems comes from studies on barley variety mixtures, in which a denser canopy structure, shading of the soil surface, and therefore reduced evaporation were observed to decrease the soil temperature (Cooper et al. 1987). Also, plant species can alleviate the microclimate for their neighbors by mediating wind, heat or photoinhibition (Wright et al. 2017). Different types of biotic facilitation are present in diverse systems but are not addressed in this study (Wright et al. 2017).

However, despite these examples of different resource partitioning and facilitation mechanisms occurring in crop systems, knowledge on the precise mechanisms that lead to overyielding in crop mixtures, and how environmental conditions can alter these mechanisms, still remains incomplete (Duchene and Vian 2017). While there is abundant evidence for the presence of complementarity effects in diverse agricultural systems, the presence of these processes alone does not guarantee overyielding.

Here, we applied ecological methods to a setting with crop species of agricultural importance by assessing to what extent CE and SE drive yield gains in crop mixtures compared with crop monocultures and how these effects are related to differences in plant functional traits and physical factors between mixtures and monocultures. We used the additive partitioning approach by Loreau and Hector (2001) to quantify CE and SE. By linking frequently used plant traits to yield gains in mixtures, we can improve our ability to predict optimal

species combinations, which can help to promote sustainable agricultural production through intercropping. We used four plant traits indicative of resource use: leaf dry matter content (LDMC), specific leaf area (SLA), carbon to nitrogen ratio (C:N ratio) and plant height, and two physical factors: PAR and volumetric soil water content (VWC). We used a structural equation model (SEM) to assess whether overyielding is driven by selection or complementarity effects or by a combination of both. Furthermore, we used this hierarchical model to understand the context dependence of the CE and SE and how this is linked to plant functional traits.

MATERIALS AND METHODS

Site description

The study was carried out in two outdoor experimental gardens in Zurich, Switzerland and Torrejón el Rubio, Cáceres, Spain. The Swiss site was located at an altitude of 508 m a.s.l. (47°23'45.3"N, 08°33'03.6"E), the Spanish site was at 290 m a.s.l. (39°48'47.9"N, 06°00'00.9"W). Switzerland is characterized by a temperate climate, Spain by a dry, Mediterranean climate. Main climatic differences during the growing season between the two sites were precipitation (Switzerland: 587 mm, Spain: 218 mm), daily average sunshine hours (Switzerland: 5.8 h, Spain: 9.6 h), while mean temperatures were comparable (average of daily mean, minimum and maximum temperature in Switzerland: 15.8, 10.9, 21.1°C; in Spain: 15.5, 9.7, 21.4°C) All climatic data are from the respective national meteorological services and are average values over the growing season (www.meteoschweiz.admin.ch, www.datosclima.es).

The experimental garden at each location covered 69.5 m², and was divided into 278 square plots of 0.25 m² and 40 cm depth. Plots were open at the bottom to allow root growth beyond 40 cm. Inside a bed, plots were separated from each other by metal frames. In Switzerland, the plots were arranged in 10 beds of 7 × 1 m, with two rows of 14 adjacent plots, resulting in 28 plots per bed. In Spain, the plots were arranged in 14 beds of 10 × 1 m, with two rows of 20 adjacent plots, resulting in 40 plots per bed. Two corner plots in each location were left empty. The plots were filled with local, unenriched agricultural soil. Soil structure and composition therefore differed between the sites. In Switzerland, soil was composed of 45% sand, 45% silt, 10% clay and contained 0.19% nitrogen, 3.39% carbon, and 333 mg total P/kg with a mean pH of 7.25. Spanish soil consisted of 78% sand, 20% silt, 2% clay and contained 0.05% nitrogen, 0.5% carbon, 254 mg total P/kg with a mean pH of 6.3.

The experimental gardens were irrigated throughout the growing season with the aim of maintaining the differences in precipitation between the two sites, but assuring survival of the crops during drought periods. In Switzerland, the dry threshold was set to 50% of field

capacity, with a target of 90% of field capacity, while in Spain the thresholds were 17% and 25% of field capacity, respectively. Automated irrigation was configured such that irrigation would start if the dry threshold was reached and irrigated until the target threshold was reached.

At each site, half of the beds were chosen randomly to be fertilized with N-P-K (1-1.7-1) while the other half served as unfertilized controls. Fertilizer was applied three times: 50 kg/ha just before sowing, another 50 kg/ha when wheat was tillering and 20 kg/ha when wheat was flowering.

Crop species and cultivars

At each site, experimental communities were constructed with eight annual crop species of agricultural interest. The crop species belonged to four different phylogenetic groups and are listed in Table S1 (Appendix S1: Table S1). The four phylogenetic groups were based on their phylogenetic distances: cereals diverged from the other groups 160 million years ago (Mya); superasterid herbs diverged from superrosid herbs including legumes 117 Mya and finally, legumes diverged from the other superrosid herbs 106 Mya (*TimeTree*). Phylogenetic distance was chosen as a criterion for functional similarity as it is often positively correlated with functional diversity and acts as a proxy to assess the impacts of species diversity on ecosystem functions (Mouquet, 2015). At both sites, we grew commercial cultivars typically used for organic farming in Switzerland (Appendix S1: Table S1). While these were bred for a Swiss climate, their cultivation in Spain demonstrated the ability of these cultivars to adapt to a climate change-type scenario, with conditions considerably drier than in Switzerland.

Experimental crop communities

Experimental crop communities at each site consisted of eight different monocultures, 24 different two-species mixtures consisting of two different phylogenetic groups and 16 different four-species mixtures consisting of four different phylogenetic groups. Every combination of the two-species mixture with two species from different phylogenetic groups and every possible four-species mixture with species from four different phylogenetic groups were planted. Each experimental community was replicated two times. The entire setup was repeated two times to allow for fertilizer treatment, resulting in 192 plots per site. Plots were randomized within each country and fertilizer treatment. Each monoculture and mixture community consisted of one, two or four crop species planted in four rows. The row order of the species was randomized. Sowing densities differed among phylogenetic groups and were based on current cultivation practice (Appendix S1: Table S1). Sowing was done by hand in early February 2018 in Spain and early April 2018 in

Switzerland. Weed plants were removed in May 2018 in Spain (85 d after sowing) and in June 2018 in Switzerland (70 d after sowing). We are aware that the use of 0.25 m² does not reflect a realistic agricultural setting. However, using small scale experiments is common in these kind of experiments (Jochum, 2020) and it allowed us to accommodate a large number of combinations and treatments that would otherwise not have been possible. Furthermore, covering only 70 m² of experimental field garden and using the same homogenized soil in all plots at each location allowed us to keep environmental conditions and climatic differences constant throughout one location.

Data collection

Leaf traits were measured at the time of flowering (Spain: May 2018, 94 d after sowing; Switzerland: June 2018, 65 d after sowing). Three individuals per crop species per plot were randomly marked and their height measured with a ruler from the soil surface to the highest green tissue. Trait measurements were done on these three marked individuals. Of each marked individual, 1 to 10 healthy leaves were sampled and immediately wrapped in moist cotton and stored overnight at room temperature in open plastic bags. For the subsequent leaf trait measurements (SLA and LDMC) we followed standard protocols (Cornelissen, 2003).

At the time of harvest (duration of growing season; Appendix S1: Table S2) all crops in each plot were harvested. The three marked individuals used for the trait measurements were collected separately, while all remaining plants per crop species per plot were pooled together. Plant shoots were cut at the soil surface and biomass and seeds were separated. The total number of individuals per crop species per plot was recorded. Fruits were air dried. Afterwards, seeds were separated from chaff with a threshing machine (in Switzerland: Alledrescher K35, Baumann Saatzeitbedarf, Germany; in Spain: Hege 16, Wintersteiger, Austria). Vegetative biomass was oven dried at 80°C until constant weight.

Interception of PAR by the plant canopy was measured weekly with a LI-1500 (LI-COR Biosciences GmbH, Germany). In each plot, three PAR measurements were taken at approximately 12:00 h by placing the sensor on the soil surface in the center of each of the three in-between rows. Light measurements beneath the canopy were put into context through simultaneous PAR measurements of a calibration sensor, which was mounted on a vertical post at 2 m above ground in the middle of the experimental garden. Fraction of photosynthetically active radiation (FPAR) (%) indicates the percentage of PAR that was intercepted by the crop canopy. VWC in the upper 6 cm of soil was measured weekly with a ML3 ThetaProbe Soil Moisture Sensor (Delta-T, Cambridge). We used a standard calibration for mineral soil. The measurements were taken in the center of each of the three in-between rows per plot. For

further data analysis, we used FPAR and VWC values from the week of leaf trait measurements (Spain: 92 d after sowing; Switzerland: 62 d after sowing).

Plant N analyses

For chemical analyses of the plant tissue we pooled the three dried leaf samples of the marked individuals per plot and per crop species. Leaf samples were ground for 20 min in 1.2 ml tubes with two stainless steel beads in a bead mill (TissueLyserII, Qiagen). Afterwards, either 100 mg (if available) or 4 mg (if the sample was too small) of ground leaf material were weighed into tin foil cups or 5 × 9 mm tin capsules and analyzed for C and N contents. The 400 large samples were analyzed on a LECO CHN628C elemental analyzer (Leco Co., St. Joseph, USA) and the 505 small samples on a PDZ Europa 20-20 isotope ratio mass spectrometer linked to a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK). Eight samples were cross-referenced on both analytical devices (Appendix S1: Fig. S1) and measured values from LECO were corrected to account for the differences between the devices (correction factors are 1.0957 for N and 1.026 for C).

Data analysis

Before data analysis, we eliminated plots with incomplete data. Due to birds foraging on seeds, substantial numbers of plots were discarded. In total, 314 plots remained, 160 in Switzerland and 154 in Spain.

To explain differences in community-level yield between mixtures and monocultures, we calculated Δyield (commonly referred to as the net biodiversity effect) as the difference between the summed community-level yields of all species in a mixture plot and the average of the mean community-level yields of all monocultures corresponding to the species in the mixture plot. The minuend and subtrahend were square root transformed to meet the assumptions of normal distribution. Therefore, Δyield compares the observed yield in the mixture with the expected yields in a mixture based on their yields in a monoculture. We quantified the net biodiversity effect (Δyield) and its two additive components, the CE and SE according to Loreau and Hector (Loreau and Hector 2001):

$$\Delta\text{yield} = N \cdot \overline{\Delta\text{RY}} \cdot \overline{M} + N \cdot \text{cov}(\Delta\text{RY}, M) \quad (1)$$

where N is the number of species in the plot. ΔRY is the deviation from expected relative yield of the species in mixture in the respective plot, which is calculated as the ratio of observed relative yield of the species in mixture to the yield of the species in monoculture. M is the yield of the species in monoculture. The first component of the net biodiversity effect equation ($N \cdot \overline{\Delta\text{RY}} \cdot \overline{M}$) is the CE, while the second component ($N \cdot \text{cov}(\Delta\text{RY}, M)$) is the SE.

All statistical analyses were performed in R version 3.6.0. (R Core Team 2019). We used general linear mixed-effects models using restricted maximum likelihood estimation to explain yield at the community level. We assessed the significance of the fixed effects using type-I ANOVA and the Satterthwaite approximation of denominator degrees of freedom (*lme4* (Bates et al. 2015) and *lmerTest* (Kuznetsova and Brockhoff 2017) packages). Yield always refers to seed yield and was log transformed. The fixed effects of the model were country (Switzerland vs. Spain), fertilizer (fertilized vs. unfertilized), species number (two vs. four) nested in diversity (monocultures vs. mixtures), and interactions among the fixed effects (except between the nested terms). Random terms were species composition and the interactions between garden bed and all fixed effects (fertilizer, legume presence, species number and diversity). To test for effects of diversity within each country, we conducted post hoc contrasts using the *emmeans* package with Tukey adjustment for multiple comparisons (Lenth 2016).

We used linear mixed-effects models to analyze treatment effects on physical factors (FPAR, VWC), community-weighted means of plant traits (SLA, LDMC, height, leaf N, C:N ratio) and the two components of the net biodiversity effect (SE, CE) with country, fertilizer, species number nested in diversity and interactions among these as fixed effects. Random terms were species composition and the interactions between garden bed and all fixed effects (fertilizer, legume presence, species number and diversity). Response variables were log transformed, except for SE and CE. Total aboveground biomass of each species was used as weights for community-weighted means of trait values (Roscher et al. 2012). These models were fitted with the “*asreml*” function in the *asreml* package in R and results were extracted using the “*test.asreml*” function in the *pascal* package. We used binomial distributions for % data (FPAR, VWC, leaf N), gamma distributions for ratios (SLA, LDMC, C:N ratio) and Poisson distribution for count data (plant height).

We constructed a piecewise SEM from the *piecewiseSEM* package (Lefcheck and Freckleton 2016) to analyze significant pathways of interactions between the parameters in our model. We started with an *a priori* model with the aim of analyzing significant pathways between physical factors, differences in plant traits between mixtures and monocultures and selection and complementarity effects (Appendix S1: Fig. S2). We then used the *dSep* function for tests of directed separation, which allowed us to re-include significant direct or indirect paths that were missing in our *a priori* model. The inclusion of each missing pathway was evaluated with the Akaike Information Criterion (AIC), to estimate the robustness of the current model compared with other models of the same dataset. Whenever the tested pathways were significant ($P < 0.05$) and the new model generated a lower AIC score, they were included. If

traits strongly correlated, we removed one of the two, keeping the one trait which would lead to the best model fit. We included bidirectional relationships between physical factors and traits as well as biodiversity effects, to account for the possibility that trait expression and biodiversity effects can be responses but also drivers of physical factors. When the final model was reached (lowest AIC score with the most variables included) we evaluated the goodness-of-fit of the model by using chi-square (χ^2) statistics (Shipley 2009, Lefcheck and Freckleton 2016). If the χ^2 result was statistically non-significant ($P > 0.05$) it indicated a good fit of the model to the data. For each variable, we reported the conditional coefficient of determination (R^2_c), which represents the variance explained by fixed and random effects.

As the additive components of the net biodiversity effect, the CE and SE, express the difference in productivity between monocultures and mixtures, we aimed to explain this difference through differences in physical and plant characteristics between monocultures and mixtures. We used a Δ to indicate differences between monocultures and mixtures. We calculated ΔVWC and ΔFPAR according to Eq. 2:

$$\Delta\text{VWC} = \overline{\text{VWC}}_{\text{mix}} - \overline{\text{VWC}}_{\text{mono}} \quad (2)$$

where $\overline{\text{VWC}}_{\text{mix}}$ is the average of all three measurements of VWC per mixture plot and $\overline{\text{VWC}}_{\text{mono}}$ the average of all three measurements of VWC of the respective monoculture plots. The same was calculated for ΔFPAR . To scale up plant trait measurements to the community level, community-weighted means in mixtures and monocultures were used to calculate ΔSLA , ΔLDMC , $\Delta\text{C:N}$ ratio and Δheight :

$$\Delta\text{SLA} = \text{CWM} \cdot \text{SLA}_{\text{mix}} - \text{CWM} \cdot \text{SLA}_{\text{mono}} \quad (3)$$

where $\text{CWM} \cdot \text{SLA}_{\text{mix}}$ is the community-weighted mean of SLA of all species in a mixture plot and $\text{CWM} \cdot \text{SLA}_{\text{mono}}$ the community-weighted mean of SLA in monoculture of all the species in the respective plot. Aboveground biomass of each species was used as weights. ΔLDMC , $\Delta\text{C:N}$ ratio and Δheight were calculated in a similar manner according to Eq. 3.

RESULTS

Response of community-level yield to treatments

Community-level yield was significantly affected by country, with 88% higher yields in Switzerland compared with Spain (Fig. 1a; Appendix S1: Table S3). Tukey post hoc tests revealed that productivity of mixtures was enhanced, particularly in Switzerland (Appendix S1: Table S4), where four-species mixtures yielded 30% more than two-species mixtures and 93% more than monocultures. Also, two-species mixtures in Switzerland yielded 48% more than monocultures. The interaction of

country \times legume indicated that mixtures with legumes yielded more than mixtures without legumes in Spain, while in Switzerland mixtures with legumes yielded less than mixtures without legumes (Fig. 1b; Appendix S1: Table S4).

Physical factors, plant traits and biodiversity effect components

Physical factors did not differ significantly between diversity treatments (Fig. 2; Appendix S1: Tables S5, S6). Limited water input resulted in a significantly lower VWC in Spain. However, VWC did not vary significantly in response to fertilizer or diversity treatments (Fig. 2a; Appendix S1: Table S6). Both plant height and FPAR were significantly higher in Switzerland than in Spain, indicating that canopy closure was more complete and that vegetative growth was generally stronger in Switzerland than in Spain (Fig. 2b, e). Plant height was significantly higher in mixtures compared with monocultures and in four-species compared with two-species mixtures (Fig. 2e; Appendix S1: Fig. S4). SLA of crops was significantly higher in Switzerland and LDMC showed an opposite behavior, with higher values in Spain than in Switzerland (Fig. 2c, d; Appendix S1: Fig. S4). Neither LDMC nor SLA responded significantly to fertilizer or diversity treatments. Leaf N did not differ between treatments (Appendix S1: Table S6). C:N ratio was significantly higher in Switzerland than in Spain (Fig. 2g; Appendix S1: Tables S5, S6). CE was stronger in Switzerland than in Spain and stronger in four-species than in two-species mixtures in Switzerland (Fig. 2h; Appendix S1: Fig. S4). SE showed no response to any treatment factor (Fig. 2i).

Structural equation model to explain community-level yields

The piecewise SEM showed a good fit to the data ($\chi^2 = 13.29$, P -value = 0.504) and explored the links between experimental treatment factors (country, fertilizer, mixture diversification), physical factors (ΔFPAR , ΔVWC) and plant traits (ΔSLA ; $\Delta\text{C:N}$ ratio, $\Delta\text{plant height}$) and finally linked these to the biodiversity effect components, CE and SE (Fig. 3).

Biodiversity effect components were negatively correlated to one another. The SE was only related to Δheight , indicating that increasing plant height in mixtures compared with monocultures increased the selection effect (Fig. 3). The CE was positively related only to ΔSLA . Increases in ΔSLA indicate larger leaf area per unit leaf dry weight in mixtures compared with monocultures, which increased the CE.

The negative correlation between $\Delta\text{C:N}$ ratio and ΔFPAR implies that leaf N contents were positively related to light interception. As more than 50% of leaf N is allocated to light-harvesting compounds (i.e. chlorophyll),

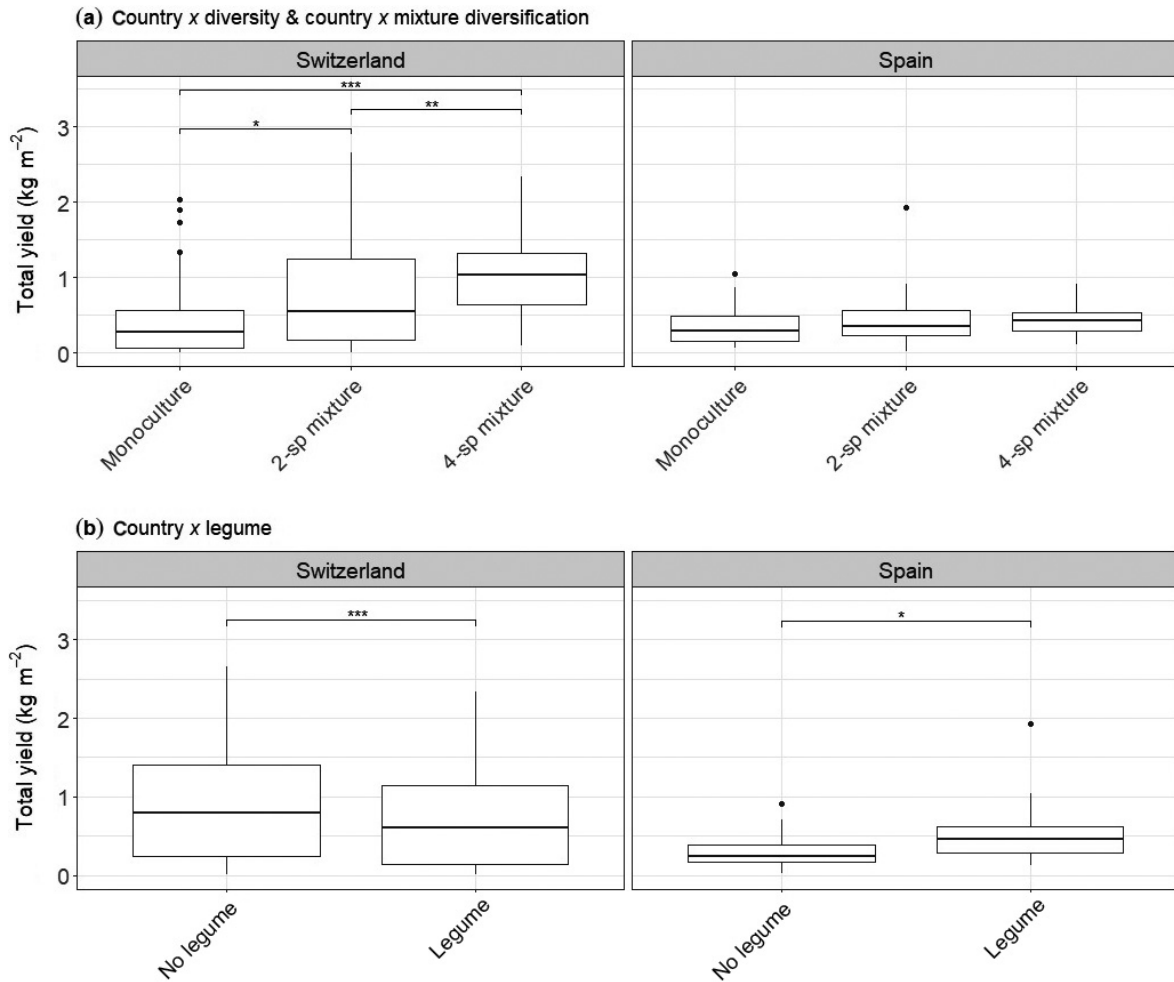


FIG. 1. Community-level yields in kg dry weight per m^2 visualizing the significant results from Appendix S1: Table S3. Differences in community-level yield between countries (a), diversity levels (a), mixture diversification (two- vs. four-species mixtures) (a), between the two-way interactions country \times legume (b), country \times diversity (a) and country \times mixture diversification (a) and country \times legume (b). Brackets indicate significant differences between treatments and labels above brackets indicate which treatment was significant at $\alpha = 0.05$ (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). $n = 314$.

increasing leaf N contents are a common response to lower light conditions (Evans and Clarke 2019). For instance, leaf N contents were observed to increase in watermelon when intercropped, compared with when cultivated as a monoculture as a response to lower light conditions in the intercrop (Franco and King 2018). The positive bidirectional relationship between Δ SLA and both physical factors, suggests that increasing light interception and soil water content increased SLA, therefore promoting larger leaf area per leaf mass, while larger SLA also led to higher light interception and soil water contents. Based on standardized effect sizes, the link between Δ FPAR and SLA was 1.7-fold stronger than the link to Δ VWC. Δ VWC and SE were positively related to each other, with the effect of Δ height being 1.2-fold stronger than the effect of Δ VWC on SE. Therefore, higher soil water contents in mixtures compared with monocultures increased the SE in mixtures compared with monocultures, while a higher SE in

mixtures compared with monocultures caused higher soil water content in mixtures compared with monocultures (Fig. 3).

Δ FPAR varied in response to fertilizer treatment, with 10% higher values in unfertilized treatments, indicating that crop mixtures intercepted more light than crop monocultures and that this effect was stronger in unfertilized treatments (Appendix S1: Table S7). Δ VWC varied significantly among countries, with more negative values in Switzerland compared with Spain. This indicates that soils in crop monocultures had a higher water content than in crop mixtures and that this effect was more pronounced in Switzerland than in Spain. Δ C:N significantly varied among countries and was 104% higher in Switzerland than in Spain (Fig. 3; Appendix S1: Table S7). Both SE and CE responded significantly to country. CE was 540% higher in Switzerland than in Spain and SE was 350% higher in Switzerland than in

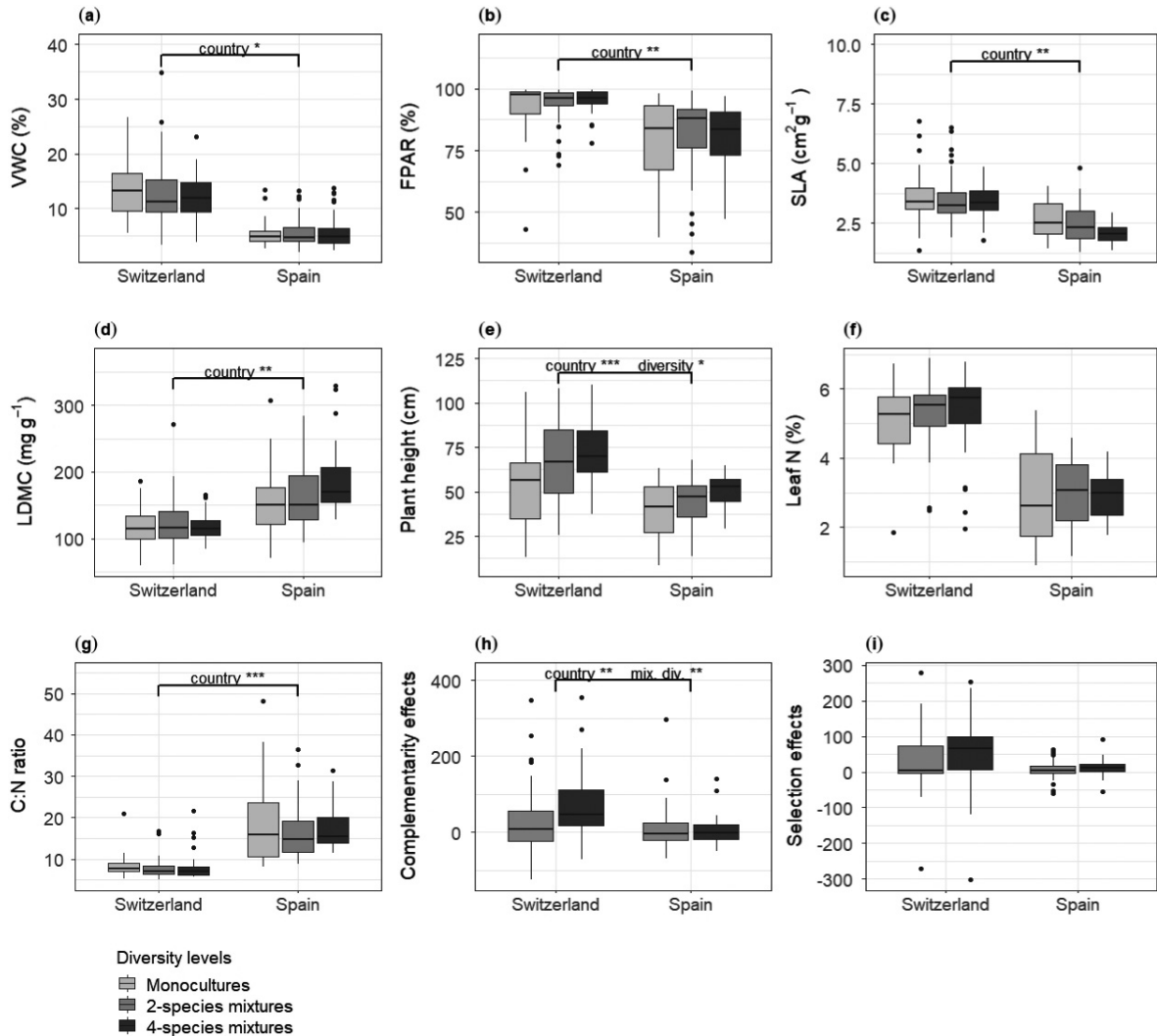


Fig. 2. Community-level means for the physical factors volumetric soil water content (VWC) (a) and absorption of photosynthetically active radiation (FPAR) (b) and community-weighted means of the plant traits specific leaf area (SLA) (c), leaf dry matter content (LDMC) (d), plant height (e), leaf N (f) and C:N ratio (g) and the biodiversity effect components, divided into complementarity (h) and selection effects (i). Data are shown for both countries and separated by levels of diversity. Complementarity and selection effects are only available for mixtures. Brackets indicate significant differences between treatments and labels above brackets indicate which treatment (mix. div. = mixture diversification) was significant at $\alpha = 0.05$ (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). $n = 314$.

Spain. CE was the only variable responding to mixture diversification and CE was 110% higher in four- than in two-species mixtures. The effect of mixture diversification on CE was as strong as the effect from Δ SLA (Fig. 3; Appendix S1: Table S7). CE and SE contributed almost equally to Δ yield (Appendix S1: Fig. S5).

DISCUSSION

Our study found increasing yields from crop monocultures to two- to four-species mixtures at the temperate site in Switzerland but not at the dry site in Spain. Community-level yield did not respond to fertilizer treatments but varied strongly between the two countries. SE

and CE were linked to differences in plant height and SLA, respectively, between monocultures and mixtures. While SLA was linked to light use and soil moisture, plant height showed no link to physical factors. The effect of mixture diversification on CE was not mediated through any of the abiotic factors or plant traits measured in this study but acted directly upon CE.

Positive biodiversity–productivity relationships are context dependent

In Switzerland community-level yield increased from monoculture to mixture and from two- to four-species mixtures, while diversity showed no effect on yield in

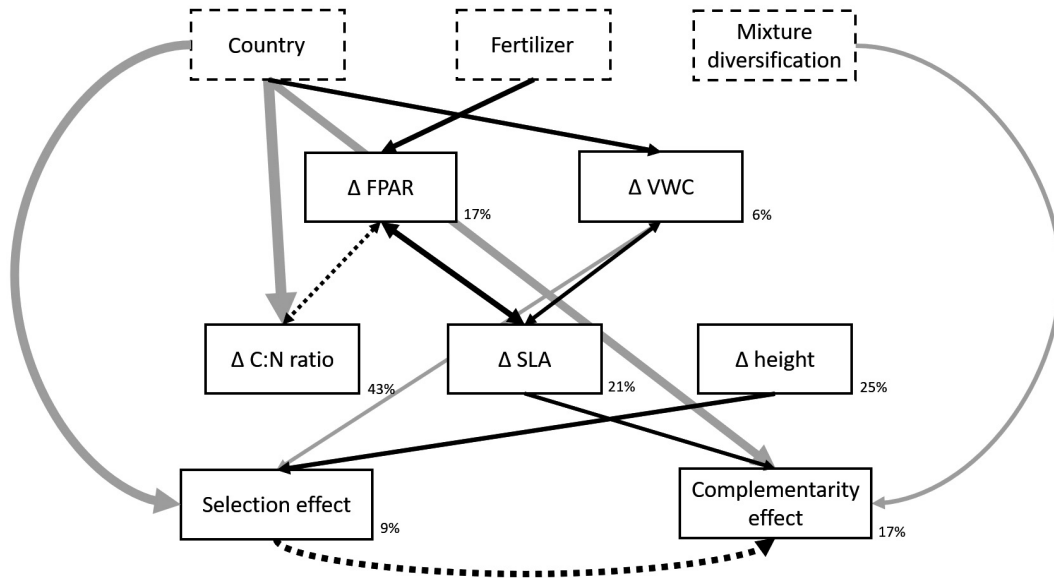


FIG. 3. Structural equation model showing the effects of experimental treatments on physical factors on plant traits and on biodiversity effect components. Δ indicates the differences between the respective measurements in mixtures compared with monocultures, therefore positive Δ values indicate higher values in mixtures compared with monocultures and vice versa. Mean values for Δ values per country, fertilizer treatment and species number are given in Appendix S1: Table S7. Displayed black arrows show significant positive (solid) or negative (dashed) relationships ($\alpha = 0.05$), gray arrows indicate the direct effects of treatment factors on traits and yield. Arrow thickness indicates effect size based on standardized path coefficients. Numbers next to the variables indicate their explained variance (R^2). Double-headed arrows indicate bidirectional relationships. Non-significant tested relationships are not shown. $n = 251$.

Spain. The differences between the two countries were diverse and included differences in precipitation and irrigation, hours of sunshine, soil nutrients, soil carbon and soil texture. Light availability and dry conditions in Spain could have been an inhibiting factor. Lower SLA values in Spain indicated that the plants had less leaf area per dry leaf mass, which could be the plants' effort to reduce leaf area exposed to high irradiance or dry conditions.

From the three growth-limiting resources, soil water and N availability were the most promising to explain the missing positive diversity–productivity relationship in Spain. Soil water content in Spain was kept low by restricting irrigation to the amount needed for plant survival. Combined with a generally drier climate in Spain, the crops were more prone to water stress. Crop yields in intercropping under drought conditions are expected to decrease (Coll et al. 2012). Also, positive diversity effects on crop water availability in intercropping remain contested and Brooker et al. (2015) suggested that these effects are limited to intercropping systems in which at least one species has a low water demand. The crop species planted in this experiment were not adapted to the dry conditions in Spain, as they were Swiss cultivars bred for use under temperate climatic conditions. A further explanation for the absence of a positive diversity–productivity relationship in Spain could be the increased allocation of C to

belowground productivity in response to dry conditions. In our study, we were interested in positive productivity effects on crop yield, therefore the focus on aboveground biomass. However, increased belowground investment can lead to a decrease in aboveground productivity, while maintaining overall community productivity (Kahmen and Perner 2005). Also, available soil water content is an important parameter controlling N_2 fixation of legumes, either directly by influencing noduleation or indirectly by reducing plant growth and therefore N_2 fixation (Sprent and Minchin 1983). However, rather than restricting N_2 fixation in Spain, the higher yield in plots with legumes compared with plots without legumes suggested the presence of a facilitative N sparing effect (i.e., an increased availability of soil N as legumes derived more N from atmospheric N_2 than soil N), which was not visible in Switzerland (Fig. 1b). However, we propose that this facilitative process was not strong enough to compensate for the difficult growing conditions in Spain. Research has shown that facilitative interactions among plants do not always increase with increasing environmental stress, particularly in arid environments (Maestre and Valladares 2005). These observations can aid the implementation of successful management strategies for mixed cropping systems under stressful environmental conditions, by suggesting that sufficient irrigation and the inclusion of a legume can improve crop growth.

Complementarity effects increased yields in mixtures compared with monocultures

In our study, CE was shown to contribute to positive biodiversity–productivity relationships in both countries (Appendix S1: Fig. S5). The CE was mainly linked to changes in SLA between mixtures and monocultures, implying that crops grown in mixtures were producing a larger leaf area per unit leaf dry mass than crops in monoculture. The increase of SLA in more diverse communities has been observed before and is achieved by an increase in leaf area through the formation of thinner leaves, therefore enabling increased light capture, which results in the often observed, more complete, canopy cover in diverse communities (Williams et al. 2020). SLA was dependent on the fraction of intercepted light and on soil water contents. The positive link between SLA and FPAR indicated that plants produced larger leaf areas per unit dry mass when less light was available. In combination with the negative link between C:N ratio and FPAR, this indicates that crops responded to a more complete canopy cover and therefore lower light access by increasing their SLA (Fig. 3) and leaf N content (lower C:N ratio) to have a larger photosynthetically active leaf area. High leaf N and high SLA are a common plant response to lower light conditions (Reich and Ellsworth 1998, Evans and Poorter 2001, Funk et al. 2017). The bidirectionality of the relationships between physical factors and plant traits indicates a positive feedback loop, in which more intercepted light and higher soil water contents increase SLA, but larger SLAs in turn increase intercepted light and soil water content.

In Switzerland, at higher mixture diversification (i.e. in four-species mixtures) the relationship between FPAR and C:N ratio became positive (Appendix S1: Table S7), indicating that, with increasing diversification, a shift in N use efficiency occurred. Therefore, four-species mixtures in Switzerland produced more yield per unit N than two-species mixtures or monocultures. Increasing N efficiency in more diverse communities has been observed before in seminatural systems (van Ruijven and Berendse 2005, Fargione et al. 2007) but, to the best of our knowledge, not in intercropping systems.

CE was the only variable responding to mixture diversification, with an increase of CE in four- over two-species mixtures. Strikingly, mixture diversification did not affect any of the physical factors or plant traits measured in this study. This suggests that the plant traits measured in this study were not able to describe how mixture diversification affects complementarity. We suspect that belowground processes are driving yield increases from two- to four-species mixtures or that dynamics of nutrients other than N, or reduced impacts of pests, were possibly playing a role. Concerning the possible belowground processes, other studies have observed that the presence of a legume can increase N or P availability in the soil surrounding its roots (Temperton et al. 2007, Zhang, 2019). In our study, this observation

could potentially be important, as all four-species mixtures contained one leguminous crop, while not all two-species mixtures did so. As an alternative to not measuring the appropriate traits, it could also be that not enough traits were measured to fully capture niche differences. As suggested by earlier studies, when linking plant traits to biodiversity effect components, a large range of traits is required to explain niche differences (Kraft and Godoy 2015, Cadotte 2017).

*Selection effects due to *Chenopodium quinoa**

In this study, SE had a similarly strong effect on yield differences between mixtures and monocultures as CE. While it is often assumed that positive biodiversity–productivity relationships are driven mainly by niche differentiation (Cardinale 2013), we show here that SE was nearly as important. The SE was linked to differences in plant height between mixtures and monocultures, therefore SE increased with increasing plant height in mixtures compared with monocultures. A relationship between plant height and SE has been observed before (Cadotte 2017, Li et al. 2020) and is probably due to plant height being related to competition for light, in which taller plants outcompete shorter plants (Westoby 1998). The observed strong SE in this study could also be akin to the strong SE observed early during a long-term biodiversity experiment, in which the SE decreased with time and eventually became negative (Fargione et al. 2007).

As the SEM linked the SE to differences in plant height between mixtures and monocultures, we concluded that one highly productive and tall-growing species, *Chenopodium quinoa*, was causing this effect (Appendix S1: Fig. S3). It has been observed before that *C. quinoa* was highly competitive (Buckland 2016). The significant treatment effect of mixture diversification on plant height, particularly in Switzerland, could represent the increased probability that four-species mixtures would include a quinoa crop compared with two-species mixtures. While half of all four-species mixtures included a quinoa crop, only one-fourth of all two-species mixtures did so.

CONCLUSION

Our study showed that crop productivity increased with diversity under temperate conditions, but only weakly when crops were grown under semiarid conditions with limited availability of water and strong irradiance, indicating that the success of intercropping is strongly context dependent. Increases in productivity in mixtures compared with monocultures were caused to almost the same extent by complementarity and SE. SE and CE were explained by different plant trait syndromes. The SE was maximized in plots with tall plants and was probably caused by one single species, *C. quinoa*, which was highly productive and tall-growing. CE was

linked to increased leaf area per unit weight, indicating that crops in mixtures increased their leaf area to improve light absorption. However, CE was also stronger in four-compared with two-species mixtures and this link was not mediated through any of the measured plant traits, suggesting that other ecological processes must have been responsible for the positive diversity effect on yield beyond two-species mixtures. This finding suggests that the drivers of diversity effects from monocultures to mixtures are not the same in two- and four-species mixtures and should therefore be targeted specifically in future studies.

ACKNOWLEDGMENTS

We are grateful to Elisa Pizarro Carbonell, Carlos Barriga Cabanillas and Anja Schmutz for their help with the mesocosm experiment, and Johan Six for comments on the experimental design. We also thank the Aprisco de Las Corchuelas and the University of Zurich for allowing us to use their experimental gardens. The study was funded by the Swiss National Science Foundation (PP00P3_170645). Authors' contributions: N.E. and C.S. conceived the study with input from L.S. and R.W.B.; N.E., L.S., and C.S. collected the data; N.E. assembled and analyzed the data with the help of C.S.; N.E. and C.S. wrote the paper. All authors discussed data analyses and results and revised the manuscript. Open Access Funding provided by Eidgenössische Technische Hochschule Zurich.

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Data (Engbersen 2021) are available on Zenodo: <https://doi.org/10.5281/zenodo.5100975>